For Reference

NOT TO BE TAKEN FROM THIS ROOM

SOME EFFECTS OF ANTIBIOTICS ON SPORULATION OF *HELMINTHOSPORIUM SATIVUM* PAMMEL, KING and BAKKE

by

E. W. B. WARD

THESIS
1954(F)
#20

Ex libris
universitatis
albertaensis

Digitized by the Internet Archive
in 2018 with funding from
University of Alberta Libraries

https://archive.org/details/ward1954

ABSTRACT

An isolate of Helminthosporium sativum Pammel, King and Bakke, has been grown under a variety of cultural conditions and considerable variability in growth and sporulation has been observed. A method is outlined whereby an attempt was made to put results on a more comparable basis than has been achieved by other investigators. It was demonstrated that in the presence of certain antibiotics spore size and the abundance of sporulation can be increased over control. Spore lengths greater than those previously reported under laboratory and natural conditions have been recorded. Of a number of bacterial isolates only one, B. polymyxa, gave stimulation of H. sativum similar to that obtained with antibiotics, while B. subtilis regularly caused the development of unusual structures in cultures of the fungus where it was present.

1954(F)
#20

THE UNIVERSITY OF ALBERTA

SOME EFFECTS OF ANTIBIOTICS ON THE SPORULATION OF *HELMINTHOSPORIUM SATIVUM* PAMMEL, KING AND BAKKE

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE

DEPARTMENT OF PLANT SCIENCE

by

E. W. B. WARD

EDMONTON, ALBERTA

OCTOBER, 1954

ACKNOWLEDGMENTS

The writer wishes to express his thanks and appreciation to Dr. A. W. Henry, under whose supervision the investigation was conducted, for suggestions and criticisms during the progress of this study, and to the National Research Council of Canada for financial assistance.

TABLE OF CONTENTS

	Page
List of Tables	i
List of Figures	iii
INTRODUCTION	1
MATERIALS	2
1. Isolation of *Helminthosporium sativum*	2
2. Bacterial isolates	3
3. Antibiotic materials	3
4. Culture materials	4
5. Mounting materials for spores	4
METHODS	5
1. Growth of treated cultures	5
2. Collection of data	10
EXPERIMENTS AND RESULTS	11
1. Establishment of a single spore culture and description of isolate	11
2. Antagonism by soil bacteria	13
3. *Bacillus polymyxa* and H.S.IIB on Wheat medium (a)	15
4. *Bacillus polymyxa* and H.S.IIB on Water Agar	16
5. Griseofulvin and H.S.IIB on Wheat medium (a)	18
6. Actidione and H.S.IIB on Wheat medium (a)	20
7. Polymyxin B and H.S.IIB on Wheat medium (a)	24
8. Actidione, H.S.IIB and Asparagine Dextrose medium	24
9. Polymyxin B, H.S.IIB and Asparagine Dextrose medium	32

TABLE OF CONTENTS (continued)

Page

10. Griseofulvin, H.S.IIB and Asparagine Dextrose medium 36

11. Bacillus subtilis, B. polymyxa, Actidione and H.S.IIB on an Oatmeal medium 36

12. Bacillus subtilis, B. polymyxa, Actidione and H.S.IIB on a Wheat medium (b) 46

13. Bacillus subtilis, B. polymyxa, Actidione and H.S.IIB on a Barley medium 51

14. a-Ketoglutaric acid, Actidione and H.S.IIB on Asparagine Dextrose medium 56

DISCUSSION 60

1. Classification and limitation of the species 60

2. Significance of the results with regard to natural conditions 68

3. Physiology of sporulation 72

CONCLUSIONS 73

BIBLIOGRAPHY 75

LIST OF TABLES

Page

TABLE (i). Relative growth of H.S.IIB in light and dark 8

TABLE I. Lengths of spores of H.S.IIB collected from plant material 12

TABLE IIA. Relative growth of H.S.IIB in the presence of a number of soil bacteria 14

TABLE IIB. Spore lengths of H.S.IIB grown together with bacteria on P.D.A. plates, under similar conditions 15

TABLE III. Relative numbers, lengths & septations of spores of H.S.IIB grown for 4 days on a wheat medium (a) seeded with B. polymyxa 17

TABLE IV. Relative numbers, lengths & septations of spores of H.S.IIB grown for 4 days on water agar seeded with B. polymyxa 19

TABLE V. Relative numbers & lengths of spores of H.S.IIB grown for 4 days on a wheat medium (a) in which various concentrations of griseofulvin have been incorporated 21

TABLE VI. Relative numbers, lengths & septations of spores of H.S.IIB grown for 4 days on a wheat medium (a) in which various concentrations of actidione have been incorporated 22

TABLE VII. Relative numbers & lengths of spores of H.S.IIB grown for 4 days on a wheat medium (a) in which various concentrations of polymyxin B have been incorporated 25

TABLE VIII. Relative numbers, lengths & septations of spores of H.S.IIB grown for 3, 4 & 5 days on an asparagine dextrose medium in which various concentrations of actidione have been incorporated. Initial pH adjusted to 5.5 with KOH. Average temperature 24.6°C 28

TABLE IX. Relative numbers, lengths & septations of spores of H.S.IIB grown for 3, 4 & 5 days on an asparagine dextrose medium in which various concentrations of polymyxin B have been incorporated. Initial pH adjusted to 5.5 with KOH. Average temperature 24.4°C 33

LIST OF TABLES (continued)

Page

TABLE X. Relative numbers, lengths & septations of spores of H.S.IIB grown for 3, 4 & 5 days on an asparagine dextrose medium in which various concentrations of griseofulvin have been incorporated. Initial pH adjusted to 5.5 with KOH. Average temperature 24.3°C 37

TABLE XI. Relative numbers, lengths & septations of spores of H.S.IIB grown for 5 days on an oatmeal medium together with Actidione, B. subtilis & B. polymyxa. Initial pH adjusted to 5.5. with HCl. Average temperature 23.5°C 42

TABLE XII. Relative numbers, lengths & septations of spores of H.S.IIB grown for 5 days on a wheat medium (b) together with actidione, B. subtilis & B. polymyxa. Initial pH adjusted to 5.5 with HCl. Average temperature 23.5°C 47

TABLE XIII. Relative numbers, lengths & septations of spores of H.S.IIB grown for 5 days on a barley medium together with actidione, B. subtilis & B. polymyxa. Initial pH adjusted to 5.5 with HCl. Average temperature 23.5°C 52

TABLE XIV. Sporulation of H.S.IIB in relation to asparagine, dextrose, a-Ketoglutarate and actidione 59

TABLE XV. Treatments in which spores tended to be longer than those collected from plant material 67

LIST OF FIGURES

Page

Figure 1. Spores of H.S.IIB from wheat medium (a) 65

Figure 2. Spores of H.S.IIB from wheat medium (a) containing 1.0 p.p.m. polymyxin B 65

Figure 3. Spores of H.S.IIB from oatmeal medium containing 1.0 p.p.m. actidione 65

Figure 4. Structures from the growth of H.S.IIB in the presence of B. subtilis on barley medium 65

INTRODUCTION

It is more than a decade since the intensive study of antibiotics got under way but most of the work carried out during this period has been concerned with the search for new substances and their application to human medicine. The economic return from this kind of work has been such that few have been given time to pause and question why these substances act as they do and even fewer to ponder if and how they act under natural conditions. An antibiotic is a substance produced by one organism which is in some way antagonistic towards another, although the term is usually restricted to fairly complex organic substances showing some specificity in their action. If antibiotics are produced by organisms in their normal habitats, (and as yet there appears to be little proof that this is the case) it might be expected that they would play a considerable role in deciding the composition of the soil microflora, which in turn would influence the local flora in general. Thus a little time spent in the study of antibiotics and antagonism in general, with respect to organisms which might be expected to come under the influence of these in nature, may not, in the limit, be completely wasted.

In the brief investigations to be outlined here a fungus, Helminthosporium sativum Pammel, King and Bakke, has been studied when grown in culture in association with various antibiotic substances and a number of bacteria. H. sativum, first described in 1910 (16), causes a number of diseases of

cereals ranging from a head blight to a foot rot, which can be severe. Both its host range and geographical range are wide, attacking most cereals and many grass species and being reported from most parts of the world where cereals are grown. It might, therefore, be expected that the fungus would be readily isolated from soil. Henry (13), however, in an investigation of Alberta soils found that this was not the case, and believed the reason to be that sporulation of H. sativum was inhibited in the soil by associated microorganisms. That both sporulation and spore morphology were somewhat variable characteristics has been indicated by a number of authors, perhaps the most frequent observation being that spores produced in culture are generally much smaller than those produced on plant material. A recent observation made at this laboratory indicated that this might not always be the case when antibiotic substances were present. It appeared possible that sporulation and spore morphology might be influenced by antibiotic substances, and this formed the basis of the investigation.

MATERIALS

1. Isolation of Helminthosporium sativum.

A number of samples of wheat showing symptoms typical of those caused by H. sativum were collected in August 1952. Portions of glumes were placed on the surface of potato dextrose agar (P.D.A.) in Petri dishes and incubated. A number of isolates believed to be H. sativum were obtained and of these

one which appeared particularly vigorous, designated H.S.IIB, was chosen for further study. Spores from this isolate inoculated into wheat plants produced symptoms typical for H. sativum.

Cultures of H.S.IIB were maintained on P.D.A. slants throughout the experiments.

2. Bacterial isolates.

A number of bacteria were isolated from soil by E. A. Peterson and were used in this study.

In addition to these Bacillus subtilis strain A.32 and an isolate of Bacillus polymyxa were also used.

These organisms were maintained on P.D.A. slants throughout the experiments.

3. Antibiotic materials.

Three antibiotics were used:

a. Actidione. A substance produced by Streptomyces griseus - a common soil actinomycete - and distributed by the Upjohn Company. This substance is not antagonistic towards bacteria, but inhibits the growth of large numbers of fungi (22). It has the empirical formula $C_{15}H_{23}NO_4$ (19). Actidione was chosen because it was available at this laboratory in fair quantity, produced by soil organisms and is antagonistic to fungi.

b. Griseofulvin. An antibiotic produced by Penicillium griseofulvum, P. janczewski and P. patulum, and isolated by Brian (3) from whom a sample of the material was

---

The correct spelling of actidione is "acti-dione".

obtained. It is inactive against bacteria, but inhibits the growth of a number of fungi. Brian (3) reported it to cause peculiar distorting effects in some fungi. The empirical formula is given by Robinson (19) as $C_{17}H_{17}O_6Cl$. This substance was studied because it is produced by a number of soil organisms and because its effects on a number of fungi have been studied.

c. Polymyxin B. Obtained as Polymyxin B sulphate from Burroughs Wellcome & Co., being one of a group of substances, obtained from Bacillus polymyxa, originally isolated by Ainsworth et al (1), in 1947. Growth of a large number of bacteria is inhibited by polymyxin B, but fungi are considered to be immune. This substance was chosen mainly for this fact, i.e. that it is not antagonistic to fungi together with the fact that it is produced by a soil organism. Chemically it is a polypeptide.

4. Culture materials.

H.S.IIB was grown throughout the experiments on "Difco Bacto-Agar" or absorbent paper discs.

Other materials incorporated in media will be indicated under the separate experiments.

5. Mounting materials for spores.

The most suitable mounting material was found to be glycerine jelly, made up according to this recipe:

1. Dissolve 1 part gelatin in 6 parts distilled water for 2 hours.
2. Add 7 parts glycerine.
3. Add 1 gram phenol crystals for every 100 grams.
4. Warm and stir for 15 minutes.

(All parts by weight)

This was found to be superior to other substances due to the fact that it was simple to use, solidified readily and, most important, because it had a clearing effect on the spores, rendering them, after 2 or 3 days, much more suitable for photography.

METHODS

1. GROWTH OF TREATED CULTURES.

The methods used in the individual experiments will be indicated, where necessary in more detail, under those experiments.

In the first experiment and in a number of preliminary experiments not recorded here, H.S.IIB was grown on various media in Petri dishes. The H.S.IIB inoculum was a disc of a uniform mat cut with a sterile cork borer. This gave a fairly uniform inoculum for a number of plates, consisting of mycelium and spores. This method had drawbacks in that:

a. due to the quantity of medium in so large a vessel as a Petri dish spore formation was slow.
b. the culture existed as a series of concentric rings at different stages of development.
c. by the method of inoculation part of the old culture

medium together with possible staling products were also transferred to the new plate.

To improve upon these the following methods were used in subsequent experiments:

a. With agar as the solidifying agent.

A shallow mould was made on a glass plate by fitting four microscope slides together. The mould was filled with the medium to be used to the level of the top surface of the slides. After the medium had solidified, discs of equal size were cut out by means of a sterile cork borer. These discs were considered to be identical in size and shape and so were directly comparable. All discs used throughout the experiments were produced in the same way. Five of these discs, all from the same mould, were placed side by side on a microscope slide and the slide was placed in a Petri dish on a double layer of filter paper. The reason for this arrangement was that it enabled all 5 cultures to be removed and examined under a microscope, if necessary, without disturbing them, and also permitted the addition of water to each dish without directly contacting the cultures. Three ml. of water were added to each dish; this amount, it was found, was just absorbed by the two filter paper sheets without leaving any standing free on the surface. The addition of a fixed quantity of water to each dish meant that the humidity in each was always the same at the commencement of an experiment. Inoculation of the discs with H.S.IIB was carried out by placing one drop of a spore

suspension in sterile distilled water from a glass rod on the surface of each disc. One spore suspension was used for a whole experiment and the same glass rod was used throughout. This gave a fairly uniform inoculum in which other contaminations were avoided.

It was found, using this method, that a uniform colony developed in some 4 or 5 days.

b. With absorbent paper discs as the solidifying agent.

Using sterilized paper discs the method was essentially similar to (a). The discs were treated by soaking in the desired culture solution and draining off the excess; they were then inoculated in the same manner as outlined above.

In both (a) and (b) the operations were carried out in a sterilized inoculating chamber.

These methods were an attempt to standardize a procedure, and appear to have been largely successful, for variation between similar discs was usually small. They made it possible to carry out the experiments in a shorter period of time, thus reducing to a minimum the influence of such external variables as humidity and temperature which, without precise equipment, do show considerable fluctuation over longer periods.

The cultures were incubated in the dark in constant temperature ovens at approximately 24°C. Temperature fluctuations from 22°C to 26°C were common, but these were not regarded as too important, for Dosdall (8), who reported 24°C as an optimum for sporulation, found very little difference between spores developed at 18°C and 24°C.

There appears to be little reference in the literature to the effect of light on the growth and sporulation of H. sativum although it has been demonstrated (14) that light is essential for sporulation of H. gramineum. In order to determine whether or not growth in the dark was introducing an additional factor H.S.IIB was grown on a number of different laboratory media in light and dark and differences in growth observed. The differences were not very marked as indicated in Table (i), but did suggest that light does have a certain effect in some metabolic process connected with the growth of the fungus. There appeared to be no marked difference in spore development.

TABLE (i). RELATIVE GROWTH OF H.S.IIB IN LIGHT AND DARK.

MEDIUM	DIAMETER OF COLONY CMS. LIGHT		DARK	
WHEAT AGAR	8.0	8.2	7.5	7.5
FLAX AGAR	8.5	8.2	7.5	7.7
NUTRIENT DEXTROSE AGAR	5.5	5.75	5.0	5.0
STARCH AGAR	4.75	4.25	4.0	3.75
PRUNE AGAR	3.0	3.0	2.25	2.5
MALT AGAR	3.0	2.75	2.75	2.0
DEXTROSE AGAR	6.75	6.75	5.5	4.75
P.D.A.	2.5	2.0	2.25	1.75

and it was decided that the small disadvantage which might result from growth in the dark was negligible compared with the difficulties to be encountered in ensuring constant and uniform illumination of a number of Petri dishes in a constant temperature chamber.

It was considered necessary that in order to place the different treatments on as uniform a basis as possible the initial pH of the media should be brought to a constant figure. A pH of 5.5 was chosen for a number of reasons. Firstly, it was found that, in general, after autoclaving for 15 minutes at 15 lbs. pressure, media containing agar, and particularly those containing sugars, developed a pH between 5 and 6. Secondly, one of the antibiotics used, (Polymyxin B), was inactivated in alkaline media while griseofulvin goes into solution more readily at a lower pH. Thirdly, a much more acid medium would prevent suitable development of bacteria, and finally, H.S.IIB grew very satisfactorily at this pH.

It was found that autoclaving lowered the pH. For example, 25 grams of Bacto-Agar in 1000 c.c. distilled water heated in a water bath until dissolved, gave a pH of 7.3. This was autoclaved the following day for 15 minutes at 15 lbs. pressure and the pH dropped to 6.3; when remelted on the third day it had fallen to 5.8. In general media were autoclaved only once and the pH adjusted after autoclaving in as sterile conditions as possible. All pH measurements were made using a Beckman pH meter fitted with external electrodes, the media containing agar being measured at 40 °C; adjustments were made with $\frac{N}{10}$ KOH or $\frac{N}{10}$ HCl.

2. COLLECTION OF DATA.

If all the spores produced in any culture could be removed, counted and measured the ideal would have been achieved, but this was not possible. However, if a definite proportion of the spores was removed and if a constant sample of these was counted, and a similar sample used for measurement and other data, then the results obtained for different treatments would be directly comparable.

In the first experiments no attempt was made to compare the quantity of spores developed under different conditions; spores were merely collected, mounted and, by means of an eye-piece micrometer, measured.

In later experiments in which some attempt had been made to standardize the cultures, the procedure outlined above was followed. The nearest to "the removal of a definite proportion of spores" was to remove as many spores as possible; presumably the same obstacles to collection of spores would recur in all cases and so a similar proportion would be collected. The spores were mounted in a constant amount of glycerine jelly, being 1 drop from the end of a glass rod, and were always covered by circular cover slips of the same size. In this way the spores in each mount were dispersed or separated to an extent proportional to the number of spores collected. A photomicrograph of a portion of this mount would also contain a number of spores proportional to the number of spores collected. These spores were counted to give a value for the number of spores produced. An enlargement of this photomicrograph was made for

purposes of spore measurement and the counting of septa. Spores were measured by means of a photograph of a stage micrometer enlarged to the same extent as the spores, and trimmed so as to serve as a rule. This gave direct measurements in classes of 10 microns.

Four discs out of the five for each treatment were used giving four sets of data which were totalled and averaged before being set down in the tables. (The fifth disc gave the operator opportunity to have a small accident without jeopardizing the whole experiment.)

EXPERIMENTS AND RESULTS

1. Establishment of a single spore culture and description of isolate.

In order to eliminate excess variation in H.S.IIB a single spore line was established, and this was used in all subsequent work. The method used was simply to make a dilute spore suspension of H.S.IIB in water-agar which was then poured into a shallow layer in a Petri dish. The spores were then sufficiently well dispersed to allow selection of one on germination. This was then transferred to a P.D.A. slant and maintained as a series of such slants, transferred frequently, throughout the experiments.

The culture usually forms a dense, dark olive green growth on P.D.A., sporulating readily, and when transferred regularly shows no tendency to sector although in old cultures white

tufts of mycelium sometimes appear and occasionally small white mushroom shaped objects develop on the surface. Conidiophores develop vertically and are usually jointed and geniculate, a spore being borne on each of the "knees". The spores are dark brown and are usually much longer than they are wide. On the medium they are usually straight, being wider towards the base than the apex, tapering slightly from the widest point towards each end where they are bluntly rounded. At the base of the spore is a scar or hilum. The spores are usually transversely septate although spores with longitudinal septa have been observed occasionally on this and other media. Rarely, Y-shaped spores with two apices have been seen; these seem to occur at random and do not appear to be associated with any particular conditions. Germination of the spores occurs from the end cells.

Inoculation of this monosporous line to wheat stems gave typical lesions. Diseased portions were collected and placed on moist filter paper; spores developing on these were removed,

TABLE I. LENGTHS OF SPORES OF H.S.IIB COLLECTED FROM PLANT MATERIAL

SPORE LENGTH CLASSES (IN MICRONS)													
20	30	40	50	60	70	80	90	100	110	120	RANGE	MODE	MEAN
2	3	8	18	14	29	65	56	34	1	2	20-120	80	78.49

mounted and measured, by means of an eye piece micrometer, and the results are given in Table I.

2. Antagonism by soil bacteria.

A number of bacterial isolates from soil which had been used in previous studies at this laboratory (17) were grown in association with H.S.IIB. 1 ml. of a suspension of each of the bacterial isolates indicated in Table IIA was added to each of two molten plates of P.D.A. and thoroughly mixed with the medium before allowing it to solidify. One of these two plates was inoculated in the centre immediately with mycelium and spores of H.S.IIB; the second was inoculated after 24 hours. A clear region or halo where there was no fungal or bacterial growth usually developed, surrounding the fungal inoculum; this was measured and the results are given in Table IIA.

It can readily be seen that all the bacteria except B. polymyxa inhibited the growth of H.S.IIB. When the bacteria were given a 24 hour start practically no growth of H.S.IIB occurred except in the presence of B. polymyxa. In the cases where the bacteria and the fungus were inoculated at the same time growth of H.S.IIB took place and at the same time inhibition of bacterial growth occurred as was indicated by the halo beyond the edge of the fungal colony. In the case of B. polymyxa growth of the fungus as well as of the bacterium continued throughout the whole plate, neither apparently having any antagonistic effect upon the other.

TABLE IIA. RELATIVE GROWTH OF H.S.IIB IN THE PRESENCE OF A NUMBER OF SOIL BACTERIA.

BACTERIUM	INOCULATED WITH H.S.IIB IMMEDIATELY		INOCULATED WITH H.S.IIB AFTER 24 HRS.	
	GROWTH OF H.S.IIB	DIAMETER OF HALO	GROWTH OF H.S.IIB	DIAMETER OF HALO
8A-50	SMALL	25 m.m.	NIL	NIL
11B-50	SMALL	20 m.m.	NIL	NIL
12A-50	NIL	NIL	NIL	NIL
13A-50	SMALL	30 m.m.	NIL	NIL
13B-50	FAIR	28 m.m.	NIL	NIL
19B-50	SMALL	17 m.m.	NIL	NIL
28-50	FAIR	38 m.m.	NIL	NIL
30-50	FAIR	35 m.m.	SMALL	NIL
32-50	FAIR	28 m.m.	NIL	NIL
40-51 *	SMALL	25 m.m.	NIL	NIL
41-51 *	GOOD	NIL	EXCELLENT (45 m.m. diameter)	NIL
42-51 *	FAIR	35 m.m.	NIL	NIL

* 40-51 - Bacillus subtilis (A.32)

41-51 - B. polymyxa (A.T.C. 7070)

42-51 - B. subtilis

Spores were collected from a number of the plates in which H.S.IIB and the bacteria were added at the same time, and the lengths of these spores compared with those from a similar plate in which no bacteria were present are given in Table IIB.

TABLE IIB. SPORE LENGTHS OF H.S.IIB GROWN TOGETHER WITH BACTERIA ON P.D.A. PLATES, UNDER SIMILAR CONDITIONS.

BACTERIAL ISOLATE	SPORE LENGTH CLASSES (IN MICRONS) 20	30	40	50	60	70	80	RANGE	MODE	MEAN
8A	33	98	53	38	27	5	-	20-70	30	37.76
11B	47	119	34	5	4	-	-	20-60	30	30.43
12A	6	74	61	40	36	20	10	20-80	30	45.10
13A	11	70	66	60	36	5	-	20-70	30	42.22
13B	11	38	81	85	26	4	-	20-70	50	43.63
CONTROL	8	36	32	44	81	38	8	20-80	60	52.15

Marked reduction in spore size is obvious in all cases compared with the control.

3. Bacillus polymyxa and H.S.IIB on Wheat medium (a).

A culture medium, designated wheat medium (a) was made up as follows:

Bacto-Agar	25 grams
Ground wheat kernels	25 grams
Chopped fresh green wheat leaves and stems	25 grams

Distilled water to make 1 litre

Autoclaved at 15 lbs. pressure for 40 minutes.

10 mls. of the molten medium was placed in a test tube and a loopful of B.polymyxa from a P.D.A. slant was thoroughly mixed with it. The inoculated medium was then spread out in a mould and allowed to set. Five discs were cut out of this mould and inoculated with H.S.IIB spores, and incubated as previously indicated. Medium to which B. polymyxa had not been added was used as a control. The numbers of spores produced, the spore lengths and septations are given in Table III. It was not possible to adjust the pH of this medium because of its consistency, but a measurement taken for water that had been mixed with it and then drained off gave a pH of 5.2.

It can be seen that although there is a very considerable reduction in spore numbers in the presence of B. polymyxa there is a very marked increase in spore size, and in septation. The number of septa developed is generally proportional to the length of the spores. The spores in the control were broad in proportion to their length, while those grown with B. polymyxa were long and narrow.

4. Bacillus polymyxa and H.S.IIB on Water Agar.

Water agar, made up with 20 grams agar per litre, was used

TABLE III. RELATIVE NUMBERS, LENGTHS & SEPTATIONS OF SPORES OF H.S.IIB GROWN FOR 4 DAYS ON A WHEAT MEDIUM (a) SEEDED WITH B. POLYMYXA

	TOTAL	SPORE LENGTH CLASSES (IN MICRONS) 20	30	40	50	60	70	80	90	RANGE	MODE	MEAN
1. B. POLYMYXA	109				3	12	20	12	1	50-90	70	69.18
2. CONTROL	688	17	81	64	12	4				20-60	30	34.66

	SEPTATION CLASSES 0	1	2	3	4	5	6	7	RANGE	MODE	MEAN
1. B. POLYMYXA					1	16	19	3	4-7	6	5.6
2. CONTROL	21	48	26	9	6	5			0-5	1	1.5

as a medium, in order to test whether agar contained sufficient nutrients for growth and sporulation. The medium was inoculated and incubated in the same way as in 3. The results are recorded in Table IV.

Growth was very slight in cultures of both treatments; there was little pigmentation and no mat developed. Spores were produced, however, sufficiently well to be collected after 4 days. They were usually light in colour and borne singly on their conidiophores. In fact, it appeared that the new spores were borne on the ends of erect germ-tubes from the spores of the inoculum, the intervening normal vegetative processes having been cut out.

It can be seen that on this medium the effect of B. polymyxa was to cut down both spore production and spore size, which could be a factor of the limited food supply.

5. Griseofulvin and H.S.IIB on Wheat medium (a).

Griseofulvin was incorporated in wheat medium (a) at concentrations of 10.0, 1.0 and 0.1 p.p.m. Solutions of griseofulvin in sterile distilled water were prepared at 100, 10 and 1 p.p.m. (Griseofulvin remained partly insoluble at the higher concentrations unless acidified with acetic acid.) From these solutions 1 ml. was transferred to test tubes containing 9 mls. molten sterilized medium, thus diluting each solution ten times and giving the required concentrations of griseofulvin. A control was prepared in the same way using 1 ml. of sterile distilled water acidified with acetic acid

TABLE IV. RELATIVE NUMBERS, LENGTHS & SEPTATIONS OF SPORES OF H.S.IIB GROWN FOR 4 DAYS ON WATER AGAR SEEDED WITH B.POLYMYXA

	SPORE LENGTH CLASSES (IN MICRONS)								
	TOTAL	40	50	60	70	80	RANGE	MODE	MEAN
1. B. POLYMYXA	63	1	4	10	8	-	40-70	60	60.87
2. CONTROL	150	2	1	10	21	12	40-80	70	68.70

	SEPTATION CLASSES									
	3	4	5	6	7	8	9	RANGE	MODE	MEAN
1. B. POLYMYXA	-	-	1	6	2	-	-	5-7	6	6.11
2. CONTROL	1	-	8	7	7	4	1	3-9	5	6.25

instead of a griseofulvin solution. The four media were then used in the same manner as in previous experiments. Spores were collected after 4 days, photographed, and counted and measured. The results are given in Table V.

At 1.0 and 10.0 p.p.m. there is an indication that spore numbers were increased while spore size was increased at 10.0 p.p.m. The spores in all treatments were dark coloured and broad in proportion to their length. A definite stunting and twisting of the hyphae could be seen, particularly at 10.0 p.p.m., where frequently the hyphae growing on the surface of the disc seemed to be composed of a series of short, fat cells - resembling a string of sausages. This is probably related to the curling effect reported by Brian (3) for this antibiotic. It was noted that spore germination and development of the treated cultures was much more rapid than in the control; spores were produced at 1.0 and 10.0 p.p.m. on the second day of incubation, whereas no spores appeared in the control until the third day.

6. Actidione and H.S.IIB on Wheat medium (a).

The experiment was carried out in exactly the same way as 5, except that only 0.1 and 1.0 p.p.m of actidione were used. The results are given in Table VI and it can be seen that although fewer spores were produced at 1.0 p.p.m. than in the control, the spores produced at both 0.1 p.p.m. and at 1.0 p.p.m. were considerably larger, the means for spore length being 10 and 18 microns higher respectively. The upper limit in

TABLE V. RELATIVE NUMBERS & LENGTHS OF SPORES OF H.S.IIB GROWN FOR 4 DAYS ON A WHEAT MEDIUM (a) IN WHICH VARIOUS CONCENTRATIONS OF GRISEOFULVIN HAVE BEEN INCORPORATED.

	GRISEOFULVIN P.P.M	TOTAL	SPORE LENGTH CLASSES (IN MICRONS) 20	30	40	50	60	70	80	90	RANGE	MODE	MEAN
1.	0.0	688	17	81	64	12	4				20-60	30	34.66
2.	0.1	689	67	132	28	13	-				20-50	30	29.46
3.	1.0	941	49	110	48	13	-				20-50	30	31.14
4.	10.0	801	5	39	32	64	16	3	-	1	20-90	50	44.75

TABLE VI. RELATIVE NUMBERS, LENGTHS & SEPTATIONS OF SPORES OF H.S.IIB GROWN FOR 4 DAYS ON A WHEAT MEDIUM (a) IN WHICH VARIOUS CONCENTRATIONS OF ACTIDIONE HAVE BEEN INCORPORATED

	ACTIDIONE P.P.M.	TOTAL	SPORE LENGTH CLASSES (IN MICRONS)									
			20	30	40	50	60	70	80	90	100	110
1.	0.0	1642	3	8	11	12	15	61	68	19	-	-
2.	0.1	1791			7	5	10	23	64	64	8	1
3.	1.0	1172				2	1	15	53	66	36	7

	RANGE	MODE	MEAN
1.	20- 90	80	69.34
2.	40-110	80-90	79.84
3.	50-110	90	87.56

TABLE VI (CONTINUED)

ACTIDIONE P.P.M.		SEPTATION CLASSES											
		0	1	2	3	4	5	6	7	8	9	10	11
1.	0.0	5	4	5	4	15	19	39	45	16	3	2	1
2.	0.1					6	10	21	41	24	13	-	1
3.	1.0					2	6	17	47	57	15	1	1

	RANGE	MODE	MEAN
1.	0-11	7	5.8
2.	4-11	7	7.0
3.	4-11	8	7.4

both cases was some 20 microns higher. The data for septations follow a similar trend. The spores of the control were usually fairly wide and dark while those from the treated cultures were narrower in proportion to their length, sometimes slightly curved and with very clear regular septations. Similar to griseofulvin there was an early stimulus to growth and sporulation compared with the control, but there were no signs of hyphal distortion or curling.

7. Polymyxin B and H.S.IIB on Wheat medium (a).

The procedure used here was identical to that in the previous two experiments except that polymyxin B was the antibiotic used. The results are indicated in Table VII. Perhaps the most noticeable fact is that at 1.0 p.p.m. spore production was more than doubled and at 0.1 and 10.0 p.p.m. it was increased by half. Differences in spore length are less marked, but at 1.0 p.p.m. the mean length is 9 microns higher than the control and the upper limit is one class higher in the treated cultures than in the control. The spores produced in the presence of polymyxin were all wide, very much wider than the control and wider than spores produced in any other experiment.

8. Actidione, H.S.IIB and Asparagine Dextrose medium.

A number of experiments were carried out in which absorbent paper discs soaked in an entirely artificial medium were used for culture purposes. The medium referred to as

TABLE VII. RELATIVE NUMBERS & LENGTHS OF SPORES OF H.S.IIB GROWN FOR 4 DAYS ON A WHEAT MEDIUM (a) IN WHICH VARIOUS CONCENTRATIONS OF POLYMYXIN B HAVE BEEN INCORPORATED

	POLYMYXIN P.P.M	TOTAL	SPORE LENGTH CLASSES (IN MICRONS) 20	30	40	50	60	70	80	RANGE	MODE	MEAN
1.	0.0	844	6	24	24	74	59	7	-	20-70	50	49.12
2.	0.1	1276	3	16	17	86	99	8	2	20-80	60	52.73
3.	1.0	1808	6	8	8	53	163	48	12	20-80	60	58.49
4.	10.0	1238	7	24	20	67	102	7	1	20-80	60	51.32

Asparagine dextrose was made up as follows:

Asparagine	2.357 grams
Dextrose	5.0 grams
KH_2PO_4	0.5 grams
$MgSO_4.7H_2O$	1.0 gram
Distilled water to make 1000 mls.	

The quantity of asparagine was chosen to give 0.5 grams nitrogen, the intention being to compare a number of nitrogen sources; this intention was not completely carried out. The quantities of the two salts are those commonly employed (15). Dextrose was used as a carbon source because this has been demonstrated (15) to be the most favourable of a number of carbon sources for growth of H. sativum. The quantity of dextrose was rather less than frequently used, providing 2 grams of carbon per litre. However, the fungus grows and sporulates well on this amount as will be observed later.

After the various components had dissolved the medium was autoclaved, and the pH adjusted to 5.5 with $\frac{N}{10}$ KOH.

Three concentrations of actidione, 0.1, 1.0 and 10.0 p.p.m. were used. These were made up as indicated earlier by preparing solutions of the antibiotic ten times the strength required and taking 1 part of each and adding it to 9 parts of the untreated medium. A control was prepared in the same way substituting sterile distilled water for the actidione solution. Any effects which the addition of these solutions might have had on the pH were disregarded. Absorbent paper

discs were soaked in each of the solutions, drained and then placed five on a microscope slide, inoculated and incubated in the manner described previously.

It seemed possible from previous experiments that in cases where treatments had caused increases in spore size the effect might be due not to any direct action of the treatments but to the rapidity of development which was noted in many cases. In other words, the difference might be due to the fact that the treated cultures had reached a state of maturity sooner than the controls. In order to check this possibility this experiment was repeated 3 times, i.e. there were 3 Petri dishes and fifteen cultures for each treatment, and these were harvested after 3, 4 and 5 days.

After 2 days there was growth in all cultures except at 10.0 p.p.m., but on the third day growth and sporulation had occurred in all treatments. The results are given in Table VIII.

In all cases there was a considerable reduction in the number of spores produced in the presence of 10.0 p.p.m. actidione and in only one case - at 0.1 p.p.m. actidione after 5 days - did any increase in number occur compared with the controls. It is apparent that as regards spore length different conclusions would be reached according to the day on which the spores were collected. After 3 days, for example, it would appear from the means that 0.1 actidione had the greatest stimulative effect, while on the fourth day 1.0 p.p.m.

TABLE VIII. RELATIVE NUMBERS, LENGTHS & SEPTATIONS OF SPORES OF H.S.IIB GROWN FOR 3, 4 & 5 DAYS ON AN ASPARAGINE DEXTROSE MEDIUM IN WHICH VARIOUS CONCENTRATIONS OF ACTIDIONE HAVE BEEN INCORPORATED. INITIAL pH. ADJUSTED TO 5.5 WITH KOH. AVERAGE TEMPERATURE 24.6°C

		ACTIDIONE P.P.M	TOTAL	SPORE LENGTH CLASSES (IN MICRONS) 40	50	60	70	80	90	100	110	120	130	140	150
3 DAYS	1.	0.0	170		7	17	16	5							
	2.	0.1	175	2	1	2	17	17	4	1					
	3.	1.0	202	2	1	8	26	18	3						
	4.	10.0	48	3	8	7	5	3							
4 DAYS	1.	0.0	624			17	45	22	16	1	1				
	2.	0.1	537		1	10	36	30	25	7	3				
	3.	1.0	449		1	9	28	20	17	12	6	3	2	1	1
	4.	10.0	51		2	1	10	7	1	1					
5 DAYS	1.	0.0	759	1	2	9	34	24	18	5	2				
	2.	0.1	1082	1	-	12	35	51	29	7	4				
	3.	1.0	477		2	6	20	24	21	6	2	4	1		
	4.	10.0	54				13	3	4	1					

TABLE VIII(CONTINUED)

ACTIDIONE P.P.M	RANGE	MODE	MEAN
1. 0.0	50-80	60	64.22
2. 0.1	40-100	70-80	74.09
3. 1.0	40-90	70	71.38
4. 10.0	40-80	50	58.85
1. 0.0	60-110	70	74.31
2. 0.1	50-110	70	79.02
3. 1.0	50-150	70	84.50
4. 10.0	50-100	70	73.18
1. 0.0	40-110	70	77.39
2. 0.1	40-110	80	79.42
3. 1.0	50-130	80	82.56
4. 10.0	70-100	70	72.38

TABLE VIII (CONTINUED)

ACTIDIONE P.P.M	SEPTATION CLASSES 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. 0.0				2	15	15	10	2	1							
2. 0.1					4	10	11	8	5							
3. 1.0			1	1	7	16	16	11								
4. 10.0			2	2	5	7	2	1	1							
1. 0.0	1	1	-	-	7	25	15	14	4							
2. 0.1	3	2	-	1	5	19	15	14	7	1						
3. 1.0		1	-	-	5	5	8	13	7	5	2	1	-	-	-	1
4. 10.0					1	2	5	3	2	1						
1. 0.0			1	2	7	13	21	9	8	-	1					
2. 0.1				2	10	22	22	24	13	2						
3. 1.0					3	16	12	16	7	4	2	1	-	1		
4. 10.0						3	8	10	5	1						

TABLE VIII (CONTINUED)

ACTIDIONE P.P.M	RANGE	MODE	MEAN
1. 0.0	3- 8	4-5	5.0
2. 0.1	4- 8	6	6.0
3. 1.0	2- 7	5-6	5.5
4. 10.0	2- 8	5	4.6
1. 0.0	0- 8	5	5.6
2. 0.1	0- 9	5	5.1
3. 1.0	1-15	7	6.9
4. 10.0	4- 9	6	6.4
1. 0.0	2-10	6	5.9
2. 0.1	3- 9	7	6.1
3. 1.0	4-13	-	6.6
4. 10.0	5- 9	7	6.7

was outstanding and by the fifth day the differences had been considerably reduced, although 5 microns still separated 1.0 p.p.m. actidione and the control. Despite the reduction in differences between the means there was little indication that the upper limit of spore length in the control would approach that obtained in the presence of 1.0 p.p.m. actidione. The upper limit found with 1.0 p.p.m. after four days is believed to be the highest ever reported. The figures for septations, in general, followed those for length, although it would appear that except at 3 days, spores produced in 10.0 p.p.m. actidione tended to have more septa in proportion to their length than did those in other treatments, which may be due to some inhibitive effect of actidione at this concentration.

9. Polymyxin B, H.S.IIB and Asparagine Dextrose medium.

The same materials and procedure as in 8 were used here except that polymyxin B was substituted for actidione. The results are given in Table IX.

After 3 days there was some indication, from the means for spore length, of an initial stimulus to increased spore size. At 4 and 5 days, however, the treatments had evened out in this respect. The spores produced in the presence of polymyxin B, as noted in an earlier experiment, were broad in comparison to their lengths, and appeared to be more heavily pigmented than the controls. Septation was very regular and, as can be seen from the table, was closely proportional to

TABLE IX. RELATIVE NUMBERS, LENGTHS & SEPTATIONS OF SPORES OF H.S.IIB GROWN FOR 3, 4 & 5 DAYS ON AN ASPARAGINE DEXTROSE MEDIUM IN WHICH VARIOUS CONCENTRATIONS OF POLYMYXIN B HAVE BEEN INCORPORATED. INITIAL pH. ADJUSTED TO 5.5 WITH KOH. AVERAGE TEMPERATURE 24.4°C

	POLYMYXIN P.P.M	TOTAL	SPORE LENGTH CLASSES (IN MICRONS) 30	40	50	60	70	80	90	100	110	120
3 DAYS	1. 0.0	136			4	16	21	9	3			
	2. 0.1	130			1	4	20	14	1			
	3. 1.0	93			3	8	14	16	1			
	4. 10.0	172			1	3	18	24	11	2		
4 DAYS	1. 0.0	205			2	2	19	25	12	1		
	2. 0.1	423		1	2	8	32	23	8	3	1	
	3. 1.0	300			2	7	19	29	9	1	1	
	4. 10.0	240		1	4	7	28	22	6	1		
5 DAYS	1. 0.0	539		2	4	14	30	66	22	7	2	
	2. 0.1	721			1	12	32	67	21	8	1	
	3. 1.0	428		1	2	11	27	40	18	4		
	4. 10.0	572	1 -		1	12	29	49	16	2	-	2

TABLE IX (CONTINUED)

POLYMYXIN P.P.M	RANGE	MODE	MEAN
1. 0.0	50- 90	70	68.30
2. 0.1	50- 90	70	72.50
3. 1.0	50- 90	80	70.95
4. 10.0	50-100	80	77.97
1. 0.0	50-100	80	77.54
2. 0.1	40-110	70	74.74
3. 1.0	50-110	80	76.32
4. 10.0	40-100	70	72.75
1. 0.0	40-110	80	77.55
2. 0.1	50-110	80	78.66
3. 1.0	40-110	80	76.80
4. 10.0	30-110	80	77.05

TABLE IX (CONTINUED)

POLYMYXIN P.P.M	SEPTATION CLASSES 3	4	5	6	7	8	9	10	RANGE	MODE	MEAN
1. 0.0	1	11	21	10	3				3- 7	5	5.1
2. 0.1		5	16	9	5	3			4- 8	5	5.6
3. 1.0	3	6	14	14	3	1			3- 8	5-6	5.3
4. 10.0	1	4	7	7	8	3			3- 8	5-6	6.0
1. 0.0	2	5	17	15	7	1			3- 8	5	5.5
2. 0.1	1	10	11	15	8	1	-	1	3-10	6	5.6
3. 1.0	1	5	17	23	12	6			3- 8	6	5.9
4. 10.0	1	6	15	11	14	1			3- 8	5	5.7
1. 0.0	3	10	18	24	20	2	4		3- 9	6	5.9
2. 0.1	1	8	14	20	12	4	2		3- 9	6	5.9
3. 1.0	1	6	13	18	7	6	2		3- 9	6	5.9
4. 10.0	3	7	17	22	7	-	2	2	3-10	6	5.7

length. Spore numbers seem to have been little influenced by the treatments although some variation did occur.

10. Griseofulvin, H.S.IIB and Asparagine Dextrose medium.

The procedure here was the same as in the previous two experiments, griseofulvin being the antibiotic used. The results are given in Table X.

The most noticeable feature is the sharp reduction in the number of spores produced in the presence of 10.0 p.p.m. griseofulvin. This provided only a small number of spores for measurement purposes, but as the mode in all three cases is the same, perhaps a fairly true picture is given. More spores were produced after 5 days with 0.1 and 1.0 p.p.m. griseofulvin than in the control, and at these concentrations there is possibly some tendency for spore size to be increased, but the differences are small.

11. Bacillus subtilis, B. polymyxa, Actidione and H.S.IIB on an Oatmeal medium.

An oatmeal medium was made up as follows:

Oatmeal	50 grams
Bacto-Agar	10 grams

Distilled water to make 1 litre.

Autoclaved for 15 minutes at 15 lbs. pressure.

The pH of the medium was adjusted to 5.5 with $\frac{N}{10}$ HCl, and 6 portions of 9 mls. each were placed in test tubes. To each of 3 of these 1 ml. of a 10.0 p.p.m. solution of actidione in sterile distilled water was added, and to each of the other 3

TABLE X. RELATIVE NUMBERS, LENGTHS & SEPTATIONS OF SPORES OF H.S.IIB GROWN FOR 3, 4 & 5 DAYS ON AN ASPARAGINE DEXTROSE MEDIUM IN WHICH VARIOUS CONCENTRATIONS OF GRISEOFULVIN HAVE BEEN INCORPORATED. INITIAL pH ADJUSTED TO 5.5 WITH KOH. AVERAGE TEMPERATURE 24.3°C

	GRISEOFULVIN P.P.M	TOTAL	SPORE LENGTH CLASSES (IN MICRONS) 30	40	50	60	70	80	90	100	110
3 DAYS	1. 0.0	75	1	1	1	7	13	10	1		
	2. 0.1	137		1	-	1	19	19	3	1	
	3. 1.0	101			4	4	18	5	1		
	4. 10.0	43	1	2	5	10	7				
4 DAYS	1. 0.0	200			4	20	19	7	1		
	2. 0.1	154			1	9	27	8	2		
	3. 1.0	200		1	2	6	24	8	4	1	
	4. 10.0	76	1	2	10	13	6				
5 DAYS	1. 0.0	465		1	2	15	34	38	12	2	
	2. 0.1	569			4	13	35	44	10	1	1
	3. 1.0	559		1	2	6	28	30	16	6	
	4. 10.0	18			1	3	-	1			

TABLE X (CONTINUED)

GRISEOFULVIN P.P.M	RANGE	MODE	MEAN
1. 0.0	30- 90	70	68.82
2. 0.1	40-100	70-80	75.46
3. 1.0	50- 90	70	68.44
4. 10.0	30- 70	60	58.00
1. 0.0	50- 90	60-70	66.28
2. 0.1	50- 90	70	70.21
3. 1.0	40-100	70	70.87
4. 10.0	30- 70	60	56.56
1. 0.0	40-100	80	74.42
2. 0.1	50-110	80	74.63
3. 1.0	40-100	80	77.22
4. 10.0	50- 80	60	62.00

TABLE X (CONTINUED)

GRISEOFULVIN P.P.M	SEPTATION CLASSES 0	1	2	3	4	5	6	7	8	9	10	11
1. 0.0	1	1	-	-	4	8	8	1				
2. 0.1					6	13	6	4	2			
3. 1.0				2	8	6	6	-	1			
4. 10.0	1	-	-	2	4	5	4	1				
1. 0.0			2	-	8	7	9	3	3			
2. 0.1					8	13	10	3	-	1		
3. 1.0				1	7	9	5	3	-	-	1	2
4. 10.0			1	-	8	6	5	1				
1. 0.0					9	25	21	28	1	6		
2. 0.1					8	22	13	9	1	1		
3. 1.0					8	18	13	7	5	1		
4. 10.0					1	2	1	1				

TABLE X (CONTINUED)

GRISEOFULVIN P.P.M	RANGE	MODE	MEAN
1. 0.0	0- 7	5-6	4.9
2. 0.1	4- 8	5	5.5
3. 1.0	3- 8	4	4.9
4. 10.0	0- 7	5	4.6
1. 0.0	2- 8	6	5.3
2. 0.1	4- 9	5	5.3
3. 1.0	4-11	5	5.7
4. 10.0	2- 7	5	4.8
1. 0.0	4- 9	7	6.1
2. 0.1	4- 9	5	5.6
3. 1.0	4- 9	5	5.7
4. 10.0	4- 7	5	5.4

1 ml. of sterile distilled water. Two of the tubes containing actidione were inoculated, one with a loopful of B. subtilis and the other with B. polymyxa. Two of the untreated tubes were inoculated in the same way. The media were held in a water bath at 40°C to prevent them solidifying. Thus there were six treatments; a control, actidione alone and combined with each of the bacteria, and the bacteria alone. The media were inoculated, incubated and the data collected in the usual manner. The incubation period was extended to 5 days in order to effect a compromise between the possibilities of harvesting the control before it was fully developed and of cancelling any early stimulating effect of the treatments by delaying collection of data until the control had "caught up". The results are given in Table XI.

No spores were produced in the presence of B. subtilis with or without actidione. No conidiophores developed and the growth was mainly on the surface of the discs. However, the growth was not normal as compared with that observed in other cultures. The mycelium itself appeared normal but it bore laterally lightly pigmented globular bodies of various sizes, usually singly but occasionally in groups. They were usually sessile on the hyphae and sometimes were present in such abundance and proximity that it was impossible to take a clear photograph of them. An example of this type of structure is demonstrated in Figure 4. Nobody, without previous information, would dare to assume, if shown one of the cultures,

TABLE XI. RELATIVE NUMBERS, LENGTHS & SEPTATIONS OF SPORES OF H.S.IIB GROWN FOR 5 DAYS ON AN OATMEAL MEDIUM TOGETHER WITH ACTIDIONE, B. SUBTILIS & B. POLYMYXA. INITIAL pH ADJUSTED TO 5.5 WITH HCI. AVERAGE TEMPERATURE 23.5°C

		SPORE LENGTH CLASSES (IN MICRONS)											
	TOTAL	20	30	40	50	60	70	80	90	100	110	120	130
1. CONTROL	1527	3	14	14	31	37	61	64	40	10			
2. ACTIDIONE 1.0 P.P.M	2637					4	25	71	109	63	14	5	1
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	NO SPORES PRODUCED												
4. ACTIDIONE 1.0 P.P.M + B.POLYMYXA	512		6	23	21	8	13	16	18	5			
5. B.SUBTILIS	NO SPORES PRODUCED												
6. B.POLYMYXA	599	1	18	20	25	17	21	15	5				

TABLE XI (CONTINUED)

	RANGE	MODE	MEAN
1. CONTROL	20-100	80	69.63
2. ACTIDIONE 1.0 P.P.M	60-130	90	88.87
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	-	-	-
4. ACTIDIONE 1.0 P.P.M + B.POLYMYXA	20-100	40	63.09
5. B.SUBTILIS	-	-	-
6. B.POLYMYXA	20- 90	30	55.33

TABLE XI (CONTINUED)

	SEPTATION CLASSES											
	0	1	2	3	4	5	6	7	8	9	10	11
1. CONTROL	2	7	12	8	17	41	27	33	17	6	1	
2. ACTIDIONE 1.0 P.P.M							13	23	29	34	10	3
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	NO SPORES PRODUCED											
4. ACTIDIONE 1.0 P.P.M + B.POLYMYXA	1	4	7	20	13	16	12	22	5	2		
5. B.SUBTILIS	NO SPORES PRODUCED											
6. B.POLYMYXA	2	10	13	21	19	36	9	8	2			

TABLE XI (CONTINUED)

	RANGE	MODE	MEAN
1. CONTROL	2-10	5	5.4
2. ACTIDIONE 1.0 P.P.M	6-11	9	8.1
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	-	-	-
4. ACTIDIONE 1.0 P.P.M + B.POLYMYXA	0- 9	7	4.8
5. B.SUBTILIS	-	-	-
6. B.POLYMYXA	0- 8	5	4.0

that it was in any way related to H. sativum.

In the other treatments there were considerable differences in the numbers of spores produced. In the presence of actidione alone over 1000 more spores were counted than in the control, while B. polymyxa reduced the numbers of spores to about 1000 less than the control. As regards spore length, the presence of actidione alone resulted in a very considerable increase both of the mean (19 microns) and of the upper limit (30 microns). B. polymyxa reduced spore length although addition of actidione resulted in an increase of spore length by about 10 microns in the mean. Septation showed the same trends as length. Curved spores were fairly common on this medium, appearing more frequently in the actidione treatments.

12. Bacillus subtilis, B. polymyxa, Actidione and H.S.IIB on a Wheat medium (b).

Wheat medium (b) was prepared as follows:

Ground wheat kernels	50 grams
Bacto-Agar	10 grams
Distilled water to make 1 litre.	
Autoclaved for 15 minutes at 15 lbs. pressure.	

Using this medium exactly the same procedure as that described under 11 was followed and the results are given in Table XII.

The results followed a similar pattern to those obtained with oatmeal medium. Rather more spores were produced in the

TABLE XII. RELATIVE NUMBERS, LENGTHS & SEPTATIONS OF SPORES OF H.S.IIB GROWN FOR 5 DAYS ON A WHEAT MEDIUM (b) TOGETHER WITH ACTIDIONE, B. SUBTILIS & B. POLYMYXA. INITIAL pH ADJUSTED TO 5.5 WITH HCl. AVERAGE TEMPERATURE 23.5°C

	TOTAL	SPORE LENGTH CLASSES (IN MICRONS) 20 30 40 50 60 70 80 90 100 110 120
1. CONTROL	1526	10 41 32 47 68 53 35 12
2. ACTIDIONE 1.0 P.P.M	2658	3 30 80 122 42 5 1
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS		NO SPORES PRODUCED
4. ACTIDIONE 1.0 P.P.M + B.POLYMYXA	1040	2 7 18 39 64 13
5. B.SUBTILIS		NO SPORES PRODUCED
6. B.POLYMYXA	1096	13 43 45 63 63 35 5

TABLE XII (CONTINUED)

	RANGE	MODE	MEAN
1. CONTROL	20- 90	60	57.69
2. ACTIDIONE 1.0 P.P.M	60-120	90	86.25
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	-	-	-
4. ACTIDIONE 1.0 P.P.M + B.POLYMYXA	40- 90	80	73.64
5. B.SUBTILIS	-	-	-
6. B.POLYMYXA	20- 80	50-60	49.18

TABLE XII (CONTINUED)

	SEPTATION CLASSES											
	0	1	2	3	4	5	6	7	8	9	10	11
1. CONTROL	2	7	10	11	12	33	11	6	2			
2. ACTIDIONE 1.0 P.P.M						2	19	27	17	4	1	1
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	NO SPORES PRODUCED											
4. ACTIDIONE 1.0 P.P.M + B.POLYMYXA					4	4	20	6	6			
5. B.SUBTILIS	NO SPORES PRODUCED											
6. B.POLYMYXA	8	24	27	19	22	49	13	2	1			

TABLE XII (CONTINUED)

	RANGE	MODE	MEAN
1. CONTROL	0- 8	5	3.1
2. ACTIDIONE 1.0 P.P.M	5-11	7	7.1
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	-	-	-
4. ACTIDIONE 1.0 P.P.M + B.POLYMYXA	4- 8	6	5.9
5. B.SUBTILIS	-	-	-
6. B.POLYMYXA	0- 8	5	3.4

presence of B. polymyxa than on oatmeal although there were still only about half the number produced in the control. The difference between the means for the control and o.1 p.p.m. actidione was about 29 microns and although B. polymyxa alone caused a reduction in spore size, combined with actidione the mean spore length was some 24 microns longer, 16 microns longer than the control. The spores were straight, and frequently quite broad. Septations were as usual proportional to spore length. No spores were produced in the presence of B. subtilis and structures similar to those described on oatmeal were observed (Figure 3).

13. Bacillus subtilis, B. polymyxa, Actidione and H.S.IIB on a Barley medium.

A barley medium was made up as follows:

Ground barley kernels	50 grams
Bacto-Agar	10 grams
Distilled water to make 1 litre.	
Autoclaved for 15 minutes at 15 lbs. pressure.	

The same procedure as in the previous two experiments was followed and the results are given in Table XIII. Unfortunately, due to an error, no results were obtained for the combination of B. polymyxa and actidione and so this is missing from the table.

This barley medium would not appear to be as favourable a medium for the sporulation of H.S.IIB as were the two preceding ones. Again no spores were produced in the presence

TABLE XIII. RELATIVE NUMBERS, LENGTHS & SEPTATIONS OF SPORES OF H.S.IIB GROWN FOR 5 DAYS ON A BARLEY MEDIUM TOGETHER WITH ACTIDIONE, B. SUBTILIS & B. POLYMYXA. INITIAL pH ADJUSTED TO 5.5 WITH HCl. AVERAGE TEMPERATURE 23.5°C.

		SPORE LENGTH CLASSES (IN MICRONS)									
	TOTAL	20	30	40	50	60	70	80	90	100	110
1. CONTROL	557	3	4	9	35	38	43	15	9	2	
2. ACTIDIONE 1.0 P.P.M	431			2	4	11	37	61	37	7	3
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	NO SPORES PRODUCED										
4. B.SUBTILIS	NO SPORES PRODUCED										
5. B.POLYMYXA	400				1	8	30	36	20	4	2

TABLE XIII (CONTINUED)

	RANGE	MODE	MEAN
1. CONTROL	20-100	70	61.96
2. ACTIDIONE 1.0 P.P.M	40-110	80	78.83
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	-	-	-
4. B.SUBTILIS	-	-	-
5. B.POLYMYXA	50-110	80	78.52

TABLE XIII (CONTINUED)

	SEPTATION CLASSES											
	0	1	2	3	4	5	6	7	8	9	10	11
1. CONTROL	1	7	5	10	21	60	21	10	7			
2. ACTIDIONE 1.0 P.P.M					4	20	26	44	16	6		
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	NO SPORES PRODUCED											
4. B.SUBTILIS	NO SPORES PRODUCED											
5. B.POLYMYXA					1	11	18	24	15	8	3	2

TABLE XIII (CONTINUED)

	RANGE	MODE	MEAN
1. CONTROL	0- 8	5	4.8
2. ACTIDIONE 1.0 P.P.M	4- 9	7	6.6
3. ACTIDIONE 1.0 P.P.M + B.SUBTILIS	-	-	-
4. B.SUBTILIS	-	-	-
5. B.POLYMYXA	4-11	7	6.9

of B. subtilis and globular bodies had developed. The number of spores collected in each of the three remaining treatments was small, the most being found in the control. However, both actidione and B. polymyxa brought about increases in spore length over the control, the mean length in both cases being increased by over 16 microns. The spores were usually narrow but frequently were curved. The septation was regular and again proportional to length.

14. a-Ketoglutaric acid, Actidione and H.S.IIB on Asparagine Dextrose medium.

Throughout the previous experiments the most consistent results had been obtained with actidione. At 1.0 p.p.m, this antibiotic gave a consistent increase in length, frequently together with an increase in the number of spores produced, on a variety of media. It seems to the writer that if a chemical substance in the environment of an organism affects the development of that organism, then the substance must be exerting its influence in one of two spheres. Either it is absorbed into the organism and becomes involved in some metabolic process or it acts outside the organism. If it is active outside the organism it could react chemically with the constituents of the environment altering them in such a way that they exert some influence on the organism, which of course only shifts the problem, or the action could be physical, affecting absorption for example. If actidione was active chemically outside the fungus it is surprizing that such

consistent results were obtained, for no known substance was consistently present in every medium with which the actidione could react. At the concentration used it would be surprizing if actidione could act physically, although this is a possibility. Thus it might be safe to conclude that the most likely sphere of action of actidione is actually inside the organism. Now spore formation is a normal activity for H.S.IIB; the effect of actidione is not to bring about something new but to amplify a process already occurring. The site of action of actidione must therefore be looked for along the chain of events that lead to spore development. This chain of events is unknown. The initiation of the chain would be expected to lie in some fundamental metabolic process, while the termination must lie in some local effect associated with differentiation of hyphal wall material. The problems presented here are, of course, colossal. One fundamental process common to all living organisms is respiration, and in the case of higher plants this occurs in association with the Krebs Tricarboxylic acid cycle. Whether this cycle operates in fungi is not certain (10) although some of the acids involved have been isolated from fungi and it seems likely that some process similar to that involved in the Krebs cycle does occur. With this in view H.S.IIB was grown on asparagine dextrose medium in the presence of actidione and the following acids in turn: pyruvic, fumaric, citric, a-ketoglutaric, oxaloacetic, succinic and malic, at $\frac{1}{100}$ molar concentrations.

Sporulation was similar in all cultures except in the presence of a-ketoglutaric acid where it was reduced, and it was decided to incorporate this in a further experiment.

In a basic solution containing 1.0 grams $MgSO_4.7H_2O$ and 0.5 grams KH_2PO_4 per litre were combined the following solutions at the concentrations shown, in the combinations indicated in Table XIV:

1. a-ketoglutaric acid 1.46 grams per litre ($\frac{M}{100}$).
2. Asparagine 2.357 grams per litre.
3. Dextrose 5 grams per litre.
4. Actidione 1.0 p.p.m.

Actually all the solutions were made up at ten times the required strength; 10 ml. portions of the solutions for each treatment were pipetted into conical flasks, the volume made up to 50 mls. with water if necessary, the pH of each of the 15 treatment solutions adjusted to 5.5, and then the volumes made up to 100 mls., to give the required concentrations of the ingredients. Sterile distilled water and, of course, sterilised glassware were used throughout, the electrodes of the pH metre were carefully washed both before and between treatments, and the whole process carried out beneath two germicidal ultraviolet lamps. The remaining portions of each treatment which were unused were left in flasks fitted with cotton plugs for several days and no contaminants developed.

Absorbent paper discs were soaked in the treatments and inoculated and incubated in the usual way. Spores were

TABLE XIV. SPORULATION OF H.S.IIB IN RELATION TO ASPARAGINE DEXTROSE, a-KETOGLUTARATE AND ACTIDIONE.

TREATMENT	ASPARAGINE	a-KETOGLUTARATE	DEXTROSE	ACTIDIONE	SPORULATION 3 DAYS	SPORULATION 5 DAYS
1.	#	-	-	-	x	xx
2.	-	#	-	-	o	o
3.	-	-	-	#	o	x
4.	-	-	#	-	xx	xx
5.	#	#	-	-	o	x
6.	#	-	-	#	x	x
7.	#	-	#	-	xxx	xxx
8.	-	#	-	#	o	o
9.	-	#	#	-	o	o
10.	-	-	#	#	xxx	xxx
11.	#	#	-	#	o	x
12.	#	#	#	-	xx	xxx
13.	#	-	#	#	xxx	xxx
14.	-	#	#	#	x	xxx
15.	#	#	#	#	xxx	xxx

Materials used in each treatment are indicated by #

No spores o Fair sporulation xx

Occasional spores x Good sporulation xxx

collected and mounted, but photographs were not taken and hence collection of data as in previous experiments has not been carried out. The results given in Table XIV are based on observations made during the growth of the fungus.

Until more complete results have been obtained from this experiment it would be inadvisable to draw any definite conclusions, but certain interesting facts stand out. Both asparagine and dextrose, in solution with $MgSO_4.7H_2O$ and KH_2PO_4, will permit fair sporulation of H.S.IIB on absorbent paper discs (Treatments 1 and 4) but sporulation is much increased when the two are present together (Treatment 7). Actidione with dextrose alone acts similarly to asparagine in this respect but has no stimulative effect when present with the latter alone. Actidione alone permits the formation of occasional spores after 5 days. In the three cases where no sporulation had occurred after 5 days a-ketoglutaric acid was present, and in all other treatments where this substance was present there was some reduction in sporulation at either 3 or 5 days or at both when compared with other treatments differing only in the absence of this component.

DISCUSSION

1. Classification and limitation of the species.

Drechsler (9) in 1923 discussed the distinctions between a number of species of Helminthosporium parasitic on grasses, and pointed out that as generic limits were only generally

defined and as much of the material shows considerable variability in any case, confusion has arisen with regard to species limits. H. sativum was first described by Pammel, King and Bakke in 1910 (16). They described the fungus as causing a blight of barley and of the organism they said, "Each conidiophore bears a large cylindrical dark brown spore, with from 7-12 divisions. The spores measure 105-130 microns by 15-20 microns." They state further that, "In culture the spores develop much more slowly than upon diseased leaves placed in moist Petri dishes", but make no further statements about the cultural behaviour of the isolate, although from their diagrams it is obvious that the authors were aware of differences in spore morphology between spores produced under more and less natural conditions. Dosdall (8) in 1923 reported on a number of experiments on sporulation of H. sativum. It was pointed out that although considerable variation occurred, spore size never attained the dimensions given by Pammel et al. The mean spore length found by this author varied from 67.75 microns on P.D.A. at 24°C to a maximum of 83.14 microns on fresh barley leaves. The longest spore was grown on P.D.A. at 14°C and measured 119 microns, but out of 3,500 spores measured under seven different sets of conditions only 22 were longer than 100 microns. That is to say, only this number reached or approached the lower limit indicated by Pammel et al. Dosdall concluded therefore that the original measurements of Pammel et al were rather large and that differences between closely

related species could only be determined if carefully controlled comparative studies were made. Stevens (21) in 1922 in describing spores collected from cultures of a Helminthosporium sp., which is presumably H. sativum, gives spore dimensions similar to those given by Dosdall and suggests that Pammel et al may have confused the dimensions with those of H. teres. Again Christensen (5) in 1922 found few spores above 100 microns in length and also indicates by his experiments the variability in spore dimensions from cultures grown under different conditions. Drechsler (9) also reported spore lengths much shorter than those described by Pammel et al and he concluded that there had been some confusion between H. sativum and H. teres. Drechsler does mention that one spore was found measuring 134 microns in length and this he regards as a maximum for the species. This author further points out that there is considerable variation in size and shape of spores according to the source of the isolate and the conditions under which it is grown. On barley, he states that the spores are usually curved, 3-10 septate, and measuring 60-120 microns in length. These he considers to be typical for he describes those collected from wheat as being atypical, being straight or irregularly curved, irregularly septate often longitudinally or nearly so, and showing much variation in width. Dimensions on wheat or under other conditions are not given.

It is apparent from the reports of previous investigators that spore length is a none too reliable feature on which to

base classification of H. sativum, and the results which have been presented have certainly supported this conclusion. Drechsler (9) states that typical spores are found on barley and measure 60-120 microns in length, and that spores found on other species are atypical. If this is the case, how can collections from wheat be diagnosed? The lengths of spores of H.S.IIB collected from wheat were 20-120 microns (Table I) in length and this does approach the figures given by Drechsler for spores from barley. However, spores grown on media have shown such striking deviations from this range that it would be difficult in some cases to assume that they were related. Consider the spores collected from wheat medium (a) after 4 days growth and shown in Figure 1 and compare these with the spores from plant material. Here the range is 20-60 microns, that is to say all the spores were below the lower limit given by Drechsler. This is also the case with spores produced in the presence of certain bacteria as indicated in Table IIB. At the other extreme collections have been made exceeding the range indicated by Drechsler. Taking the extreme example, the spores produced in the presence of 1.0 p.p.m. actidione (Table VIII) had a range of 50-150 microns. Nobody would readily assume that these spores belonged to the same species as those in which the range was 20-60 microns. Without previous information one might be inclined to ascribe the spores with a range of 50-150 microns to H. monoceras which Drechsler describes as having spores 40-150 microns in length and being otherwise similar to H. sativum.

Brown and Miller (4) devote a little space to discussion of species concepts in H. sativum and H. victoriae Meehan and Murphy. These authors took samples of spores from the type specimen collected by Bakke in 1909. They found the spores to measure 90-120 microns in length and were mostly curved. Thus the dimensions were lower than those given by Pammel et al (16), but not as much so as many authors would have imagined. Two isolates of H. sativum and two of H. victoriae are compared with each other and with the type with respect to spore morphology. They found that the appearance of the spores of the H. sativum isolates were similar to those drawn by Pammel et al (16), except in size, being straight, thick walled, and widest below the middle, but were not similar to the type specimens which they examined. The latter were generally thin walled, narrow, curved and widest at the middle; in fact the spores of the type more closely resembled the spores from the two isolates of H. victoriae. They state that the isolates of H. sativum and H. victoriae gave constant spore characters throughout their investigations, the sativum isolates being grown on both oats and barley. It is perhaps interesting, therefore, that on oatmeal medium in the presence of 1.0 p.p.m. actidione (see Table XI) a large number of the spores collected were curved and narrow. A number of these spores are shown in Figure 3. For one of their isolates of H. victoriae Brown and Miller show that about 50% of spores were curved, the remainder being straight, and from Figure 3 it can be seen that similar proportions of curved and straight spores are present there.

Figure 1 (upper left): Spores of H.S.IIB from wheat medium (a). (Table III)
Figure 2 (upper right): Spores of H.S.IIB from wheat medium (a) containing 1.0 p.p.m. polymyxin B. (Table VII)
Figure 3 (lower left): Spores of H.S.IIB from oatmeal medium containing 1.0 p.p.m. actidione. (Table XI)
Figure 4 (lower right): Structures from the growth of H.S.IIB in the presence of B. subtilis on barley medium. (Table XIII)

A number of authors have concluded that spores collected from cultures in the laboratory are small compared with those from plant material. Suggestions as to the reasons for this have not been made although it would appear that certain factors must be present or lacking in cultures preventing the growth of spores to their full size. In the results reported here a number of exceptions have been found to these conclusions. The data from Table I show that spores of H.S.IIB collected from plant material measured 20-120 microns in length with a mode at 80 microns and a mean of 78.49 microns; the occasions on which these dimensions were surpassed are indicated in Table XV.

Actidione, and usually at 1.0 p.p.m., is the substance most consistently present where this effect occurs, but that it is not an effect restricted solely to this substance is indicated by the fact that it occurs once in the presence of polymyxin B and once in the presence of B. polymyxa. With reference to B. polymyxa it might be mentioned here that consistent results should not be anticipated using this living organism. Assuming for example that it exerts its influence, either stimulative or inhibitive, through the production of antibiotic substances, whether or not these act in the one direction or the other may depend upon their concentration. Actidione, for example, becomes most inhibitive at concentrations much above 10.0 p.p.m. The concentration of any antibiotic substances produced would depend upon the size of the bacterial population, which in turn would depend on the suitability of the medium, and also the medium would, by its composition,

TABLE XV. TREATMENTS IN WHICH SPORES TENDED TO BE LONGER THAN THOSE COLLECTED FROM PLANT MATERIAL

TREATMENT	MEDIUM	RANGE	MODE	MEAN	TABLE
	PLANT MATERIAL	20-120	80	78.49	I
ACTIDIONE 0.1 P.P.M	WHEAT MEDIUM (a)	40-110	80-90	79.84	VI
ACTIDIONE 1.0 P.P.M	WHEAT MEDIUM (a)	50-110	90	87.56	VI
ACTIDIONE 0.1 P.P.M 4 DAYS	ASPARAGINE DEXTROSE	50-110	70	79.02	VIII
ACTIDIONE 0.1 P.P.M 5 DAYS	ASPARAGINE DEXTROSE	40-110	80	79.42	VIII
ACTIDIONE 1.0 P.P.M 4 DAYS	ASPARAGINE DEXTROSE	50-150	70	84.50	VIII
ACTIDIONE 1.0 P.P.M 5 DAYS	ASPARAGINE DEXTROSE	50-130	80	82.56	VIII
POLYMYXIN B 0.1 P.P.M 5 DAYS	ASPARAGINE DEXTROSE	50-110	80	78.66	IX
ACTIDIONE 1.0 P.P.M	OATMEAL MEDIUM	60-130	90	88.87	XI
ACTIDIONE 1.0 P.P.M	BARLEY MEDIUM	40-110	80	78.82	XIII
ACTIDIONE 1.0 P.P.M	WHEAT MEDIUM (b)	60-120	90	86.25	XII
B. POLYMYXA	BARLEY MEDIUM	50-110	80	78.52	XIII

directly influence the amount of antibiotic produced. Thus the fact that stimulative effects do occur in the presence of B. polymyxa, and these have been noted 3 times (Tables IIA, III and XIII), indicates that if the correct conditions were found this effect could be regularly produced. It would, of course, be going much too far to suggest that, because two known antibiotics can, in the laboratory, stimulate spore length to that which is found in the field, spore size therefore must be dependent on the presence of these two substances. It would be difficult to imagine any antibiotic being produced in the soil in sufficient quantity to be absorbed by a plant and be active at 1.0 p.p.m. in regions where fruiting of a parasite such as H. sativum would occur (commonly on the heads of cereals). That actidione and polymyxin have these effects in the laboratory may or may not indicate that they have some specific effect on spore size. The effect of these two substances may be a general one and at a site far removed from that of spore development, such that a host of other materials acting at different points could bring about the same end result. Alternatively, these two substances could be acting specifically, or more likely they could possess certain characteristics which may be responsible for the effects they produce. Both are nitrogen containing compounds, although unrelated.

2. Significance of the results with regard to natural conditions.

Three purified antibiotics have been used in the experiments

described here. All three are unrelated chemically. Two, actidione and griseofulvin, are described as antifungal (17) while the third, polymyxin B, is described as antibacterial, having no antifungal properties. It is therefore interesting that all three substances have some effect on sporulation of H.S.IIB, particularly in that this effect is, under certain conditions, stimulative. The effect of polymyxin B differs somewhat from that of the other two in that not only does it increase the length of the spores but also very considerably increases the breadth; compare for example Figures 2 and 3. It is possible that under natural conditions there are a number of agents which could act in a way similar to that in which these have acted in the laboratory. That B. polymyxa can have a stimulative effect under a number of conditions indicates further that this could be so, and a number of workers have reported agents of a biological nature which influence the development of H. sativum in the field. Henry (12) found that the association of soil microorganisms with H. sativum in soil greatly reduced the pathogenicity of the latter towards wheat seedlings. Greaney and Machacek (11) reported that Cephalothecium roseum Corda was antagonistic towards H. sativum, and showed that the staling products of this fungus inhibited the growth of H. sativum in culture. Sanford and Cormack (20) tested the effects of a number of isolates of Penicillium, Actinomyces and a number of other miscellaneous fungi on the virulence of H. sativum on wheat seedlings in sterile soil. Some were found to be suppressive,

others without effect, while still others increased the virulence of H. sativum. Anwar (2) reported that H. sativum was inhibited in soil and found a large proportion of organisms isolated from soil to be antagonistic towards it, including B. subtilis. Thus in general it would appear that H. sativum is an unsuccessful competitor in soil. This type of thing has been recorded in the case of other fungi. Dobbs and Hinson (7) carried out investigations on certain soil fungi, with respect to their spore germination, survival and sporulation. Their findings indicated that although it is possible to isolate a large number of fungi from soil it is difficult to demonstrate that these fungi were active in the soil and not dormant. It was demonstrated that of all spores buried in the soil by hand none that were in actual contact with the soil germinated. This inhibitive effect was removed in sterilized soil. It is suggested that, if these organisms grow at all in soil, and many undoubtedly do, they do so in small pockets, where inhibitive influences are not prevalent. Some support for this suggestion may be found in the results recorded here, for if the inhibitive effect is an antibiotic one as has been intimated by some of the authors mentioned, then at low concentrations antagonistic substances may become stimulative. Variation in concentration of such materials could be expected in soil where such factors as moisture show very considerable changes over periods of time. There are indications (Table IIA) that H.S.IIB is antagonistic towards a number of soil bacteria under some conditions, and in addition B. polymyxa has been

demonstrated as being synergistic towards H. sativum under some conditions and this, together with the fact that Sanford and Cormack (20) observed some organisms which acted in the same way, suggest also that there may be certain conditions of the soil microflora favourable to the development of H. sativum.

The reaction of H.S.IIB to B. subtilis is interesting; an example of the effect is shown in Figure 4. If this organism can alter the morphology of H.S.IIB to such an extent in culture it is possible that similar effects may occur in nature and growth in the soil, if it occurs at all, may be in a form very dissimilar to that which has been observed in the laboratory. The consistency with which this type of growth occurred when B. subtilis was present, suggests that association of these two organisms in nature might result in a similar type of growth. This might account for the rarity with which spores of H. sativum have been found in soil although it would not account for the fact that H. sativum is seldom isolated from soil as indicated, for example, by Henry (13), unless, of course, this growth form was incapable of surviving the drastic change from soil to culture medium. Other authors have described peculiar effects in H. sativum when grown in the presence of other organisms. Porter (18) describes effects similar to those mentioned here produced in the presence of an unidentified bacterium, while Christensen and Davies (6) describe saltants produced in response to Bacillus mesentericus.

3. Physiology of sporulation.

A number of reports have appeared in the literature on sporulation of fungi in general. All indicate that this process is influenced by a large number of factors, suggesting that the study is still in the peripheral stages. For H.S.IIB further factors have been demonstrated here. In Table III it was indicated that a-ketoglutarate reduces sporulation of H.S.IIB, and in higher plants this substance is associated with nitrogen metabolism. It is also a participant in the Krebs tricarboxylic acid cycle. The antibiotics which have been shown to stimulate sporulation are at higher concentrations (except polymyxin B) markedly inhibitive, and it might be possible that their effect is due to an interference with normal metabolism such that the life cycle is accelerated. Fruiting is frequently associated with decadence and these substances may bring to a more rapid halt certain processes on which a-ketoglutaric acid has a perennating effect. This is rather far above ground; in subsequent work these possibilities could be checked. Known respiratory inhibitors could be used and their effect compared with actidione, for example. Observations on the effect of actidione on respiration of H. sativum might also yield interesting results. By these two methods it should be possible to determine whether actidione is active in respiration and whether spore formation is influenced or is incident on changes in respiration. An attempt could be made to determine whether the substances have a local action

influencing the differentiation of hyphal material.

CONCLUSIONS

The reports of previous investigators with respect to variability of H. sativum have been amply confirmed by the results recorded here. Greater variation in spore length has been observed than has been reported previously and the upper limit must be extended to somewhere close to 150 microns (approximately 148 microns). It is noteworthy that although most authors conclude that spores produced in culture are generally smaller in size than those gathered from plant materials, spores exceeding those previously collected under any conditions have been obtained here from a medium, purely synthetic apart from highly purified absorbent paper discs. Curved spores, normally only described from plant material, have also been found in culture. All three antibiotics used have been shown to have some stimulative effect on spore size in culture, and as these are widely different substances both chemically and biologically it seems highly probable that many other antibiotic substances will act in the same manner at low concentrations. Where stimulation in the presence of antibiotics occurs it appears that the process of germination and events leading to sporulation are much hastened. The two bacteria used here have both shown suppression of sporulation, and one (B. polymyxa) under some conditions has shown stimulation. The effect of B. subtilis in causing an unusual growth habit of H.S.IIB may possibly be duplicated under natural conditions by this and

other organisms, and may in part account for the infrequency with which spores of H. sativum are found in the soil. The occasional stimulative effect of B. polymyxa indicates that there may be conditions in the soil where germination and sporulation of H. sativum may be stimulated.

Finally, with respect to sporulation in general, it may be said that spore development is influenced very considerably by various cultural conditions, and although antibiotics have been shown to have stimulative effects, other substances such as a-ketoglutaric acid may have a delaying effect on sporulation. However, much further work is required before any conclusions can be drawn as to the reasons for the stimulative action of the antibiotics studied.

BIBLIOGRAPHY

1. AINSWORTH, G.C., BROWN, A.M., and BROWNLEE, G. Aerosporin, an antibiotic produced by Bacillus aerosporus Greer. Nature 160:263. 1947.

2. ANWAR, A.A. Factors affecting the survival of Helminthosporium sativum and Fusarium lini in soil. Phytopathology 39: 1005-10019. 1949

3. BRIAN, P.W. Studies on the biological activity of griseofulvin. Ann. Botan. N.S.13: 59-77. 1949

4. BROWN, A.R., and MILLER, J.H. A comparative study of Helminthosporium sativum Pam., King and Bakke, and H. victoriae Meenan and Murphy on oats. Agronomy Journal 46: 63-67. 1954

5. CHRISTENSEN, J.J. Studies on parasitism of Helminthosporium sativum, University of Minnesota Agricultural Experiment Station. Technical Bulletin 11: 1922.

6. CHRISTENSEN, J.J., and DAVIES, F.R. Variation in Helminthosporium sativum induced by a toxic substance produced by Bacillus mesentericus. Phytopathology 30: 1017-1033. 1940.

7. DOBBS, C.G., and HINSON, W.H. A widespread fungistasis in soils. Nature 172: 197-199. 1953.

8. DOSDALL, L. Factors influencing the pathogenicity of Helminthosporium sativum. University of Minnesota Experiment Station, Technical Bulletin 17. 1923.

9. DRECHSLER, C. Some graminicolous species of Helminthosporium I. J. Agr. Research. 24: 641-739. 1923.

10. FOSTER, J.W. "Chemical activities of fungi" Academic Press Inc. 648 pp. 1949.

11. GREANEY, F.J., and MACHACEK, J.E. Studies on the control of root rot diseases of cereals caused by Fusarium culmorum (W.G.Sm.) and Helminthosporium sativum P. K. & B. II. Pathogenicity of Helminthosporium sativum as influenced by Cephalothecium roseum Corda, in greenhouse pot tests. Sci. Agr. 15: 377-386. 1935.

12. HENRY, A.W. The natural microflora of the soil in relation to the foot-rot problem of wheat. Can. J. Research 4: 69-77. 1931.

13. HENRY, A.W. Occurrence and sporulation of Helminthosporium sativum P. K. & B in the soil. Can. J. Research 5: 407-413. 1931.

14. HOUSTON, BYRON R., and OSWALD, JOHN W. The effect of light and temperature on conidium production by Helminthosporium gramineum in culture. Phytopathology 36: 1049-1055. 1946.

15. LILLY, V.G., and BARNETT, H.L. Physiology of the fungi. McGraw-Hill Book Company, Inc. 464 pp. 1951.

16. PAMMEL, L.H., KING, C.H., and BAKKE, A.L. Two barley blights, with comparison of species of Helminthosporium upon cereals. Iowa State College of Agricultural and Mechanic Arts, Experiment Station. Bulletin 116: 178-190. 1910.

17. PETERSON, E.A. Private communication.

18. PORTER, C.L. Concerning the character of certain fungi as exhibited by their growth in the presence of other fungi. Am. J. Botan. 11: 168-188. 1924.

19. ROBINSON, F.A. Antibiotics. Pitman. 132 pp. 1953.

20. SANFORD, G.B., and CORMACK, M.W. Variability in association effects of other soil fungi on the virulence of Helminthosporium sativum on wheat seedlings. Can. J. Research (C) 18: 562-565. 1940.

21. STEVENS, F.L. The Helminthosporium foot-rot of wheat, with observations on the morphology of Helminthosporium and the occurrence of saltation in the genus. Illinois Natural History Survey Bulletin 14 (5): 77-184. 1922.

22. WHIFFEN, A.J. The production, assay and antibiotic activity of actidione, an antibiotic from Streptomyces griseus. J. Bact. 56: 283-291. 1948.

B29769